



Atomic Energy
Control Board

P.O. Box 1046
Ottawa, Canada
K1P 5S9

Commission de contrôle
de l'énergie atomique

C.P. 1046
Ottawa, Canada
K1P 5S9

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DOSE-LETHALITY RELATIONSHIPS OF
ACUTE EXPOSURE TO ANHYDROUS AMMONIA

by

F.R. Engelhardt and M.C. Holliday
Michael Holliday & Associates

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ABSTRACT

The report reviews the acute effects of exposure to ammonia in laboratory animals and humans. From this review, a dose-lethality curve (presented as LC₅₀ vs. time) for human exposure to the gas is derived. Sufficient information on which to base a human dose-lethality relationship is available only from LC₅₀ data experimentally determined for rats and mice: it is concluded that the human response more closely follows that of the mouse. For risk-assessment modelling purposes, it is suggested that human LC₅₀ values for short exposure times (six seconds to two minutes) be considered constant (at 30,000 ppm); while at longer exposures, the logarithm of LC₅₀ varies inversely with time (leading to a value of 3,700 ppm at two hours). It is further suggested that human LC₁₀ values are half of the corresponding LC₅₀s. The derived human dose-lethality relationship is in close agreement with estimates obtained recently by other investigators.

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RÉSUMÉ

Le présent rapport est une étude des effets particuliers de l'exposition des humains et des animaux de laboratoire à l'ammoniaque. Dans le cadre de l'étude, on établit une courbe dose-léthalité (présentée sous forme de CL₅₀ par rapport au temps). La documentation sur laquelle peut s'appuyer une relation humaine dose-léthalité n'est disponible que d'après des données expérimentales de CL₅₀ chez les rats et les souris. On conclut que la réponse humaine suit de beaucoup plus près celle de la souris. À des fins de modèles de risque-évaluation, on propose que les valeurs humaines de CL₅₀ pour un temps d'exposition relativement court (de six secondes à deux minutes) soient utilisées comme une constante (à 30 000 ppm) tandis qu'avec un temps d'exposition plus long, le logarithme du CL₅₀ varie inversement avec le temps (donnant une valeur de 3 700 ppm à deux heures). On propose également que les valeurs humaines de CL₁₀ sont la moitié des valeurs correspondantes de CL₅₀. La relation dose-léthalité humaine qui en découle suit de près l'estimation obtenue récemment par les autres chercheurs.

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1. INTRODUCTION

Ammonia is recognized as a highly alkaline and acutely irritating gas which affects the respiratory tract, eyes, and exposed skin. Short exposures to high concentrations, probably of the order of thousands to tens of thousands of parts per million (ppm),* can cause death through extreme pulmonary complications. These potentially lethal properties have been known for centuries. Indeed, the toxic effects of ammonia were recognized and reported even before its identity as an individual chemical was known. Ammonia was prepared for the first time as a free gas by Priestly in 1774 and in 1805 was shown to have the molecular formula, NH_3 [1]. Lehmann, writing in 1886, cites a case reported by Ramazzini in 1718 in which ocular damage occurred in a sewer worker who was exposed to "sharp vapour", which Lehmann identified as ammonia [2].

Ammonia is widely employed as a primary industrial chemical in chemical manufacturing, metal refining, and as a fertilizer [3]. It is a gas at room temperature (boiling point, -33.4°C at atmospheric pressure), is less dense than air and is extremely soluble in water (commercial aqueous ammonia contains 28% by weight NH_3). The commercial use of direct concern to the Atomic Energy Control Board is the use of ammonia in the refining of uranium. In this process, large quantities of anhydrous ammonia (stored in pressurized containers) are used as a source of hydrogen (ammonia dissociates to nitrogen and hydrogen at high temperatures) to reduce uranium trioxide to uranium dioxide (the ultimate uranium oxide before hydrofluorination and final reduction to uranium metal) [4].

* We shall, in general, use units of parts per million (ppm) when describing atmospheric concentrations of ammonia. The factor to convert ppm to $\text{mg NH}_3/\text{m}^3$ is 0.696 at 25°C and one atmosphere pressure (101.3 kPa); the conversion factor increases in value as the ambient temperature decreases and is, for example, 0.760 at 0°C .

As part of their consequence assessment pertaining to uranium manufacturing, the Atomic Energy Control Board requested that we attempt to derive, from a comprehensive evaluation of published material dealing with ammonia toxicity in humans and animals, dose-lethality relationships for human exposure to the compound for periods ranging from six seconds (one breath) to two hours. Specifically, we were asked to derive LC₅₀ and LC₁₀ values to cover this exposure period. These lethal concentrations can be used in a consequence-assessment model developed for the Board by Monserco Limited [5]. This mathematical model can be used to predict atmospheric ammonia concentrations downwind of a massive release of the gas, such as from the catastrophic rupture of an ammonia storage tank at a uranium refinery. The model also takes into account the effect of short-term peak concentrations of the gas. Provided that the variation of LC₅₀ and LC₁₀ values are known with respect to duration of exposure, the model can be used to obtain an estimate of the number of deaths that could potentially be expected among a human population in the vicinity of an ammonia storage tank if the tank ruptured. It is worth noting that in a recently published report, Risk Analysis of Six Potentially Hazardous Industrial Objects in the Rijnmond Area: A Pilot Study [6], ammonia was one of the industrial chemicals for which a risk assessment was attempted. The utility and limitations of such physical models for risk and consequence assessment are well described in this report.

The appropriateness of such derived values for lethality is much dependent on the completeness of background information on accidental exposure conditions, the ability to extrapolate human responses from animal data, and an adequacy of experimental design in the generation of such animal data. The greater the number and importance of critical assumptions in input factors for the derivation of lethality concentrations, the greater the degree of uncertainty associated with these. The exercise which follows in this report has striven to minimize the level of assumptions as much as possible by seeking correlations among the various exposure incidents and experiments reported in the available literature.

2. HEALTH EFFECTS

The focus of the following material is on acute health effects resulting from short-term exposure to ammonia gas. Generally, exposures of less than a few hours duration are considered here. The effects of long-term exposure to ammonia, including the truly chronic exposures of weeks, months, or longer, are discussed only briefly in order to round out the clinical picture of ammonia effects.

2.1 OVERVIEW

In general, the most life-threatening effect is on the respiratory system, although skin and eyes may also be debilitated when exposed to relatively high concentrations of ammonia. Ammonia acts as a primary irritant and is similar in this respect to nitrogen dioxide or ozone. Acute exposure to such irritant gases can cause edema and epithelial necrosis, followed by purulent inflammation of the lung. Pulmonary fibrosis and emphysema may be longer term responses in these instances. Additionally, it has been demonstrated that an acute constriction of the upper respiratory passages can occur upon exposure to high concentrations of ammonia. A more physical and topical effect is the freezing of exposed tissues when a victim is exposed to liquid ammonia, for example, caused by close proximity to a pressurized ammonia container at the moment of rupture.

Reference to results obtained from laboratory animals is necessary to interpret or extrapolate to human responses, particularly because there are no reports of human exposures which enable documentation of the concentrations of ammonia to which the victims were exposed. The interpretation of animal data is complicated by experimental design and protocol, and by the limitations inherent in using an animal model for characterizing the effects of inhaled toxicants. Respiratory behaviour, morphology and physiology may differ in one or several aspects among laboratory animal species and humans [7]. Physiological differences expressed by such indices as basal

metabolism, frequency of respiration, surface of lungs, ventilation rate, and circulatory rate, as well as indices mathematically derived from these, can all be expected to influence the toxicity of inhaled ammonia. Morphological differences (e.g., the fact that rats and guinea pigs have more highly developed nasal turbinates and consequently more mucous surface area than man) would influence the initial effect of ammonia when inhaled through the nose. From the point of respiratory anatomy, none of the standard laboratory species are analogous to man (the horse, a species seldom used experimentally, most closely resembles man in pulmonary structure [7]). Physiologically there is no directly comparable system between the laboratory species and humans.

Expression of the toxicity of ammonia depends on concentration and duration of exposure (Table 2-1). One can identify four stages in the human response to exposure to high concentrations of ammonia; each of these is implicitly life threatening:

- Phase I acute obstruction of upper ventilation pathways caused by laryngo- and bronchospasms;
- Phase II acute changes in gas transfer across alveolar membranes as a result of massive pulmonary edema and extravasation;
- Phase III secondary bacterial infection leading to broncho- and pulmonary pneumonia; and
- Phase IV latent mortality due to progressive obstructive lung disease.

The timing of these phases cannot be clearly quantified because of the differential effects of ammonia concentrations and individual susceptibility. But a phased trend is observable:

- Phase I within seconds to minutes of exposure;
- Phase II within minutes during and several hours after exposure -- may overlap with Phase I;
- Phase III within a week to two months of exposure; and
- Phase IV months to years after exposure.

TABLE 2-1 Summary of Concentration and Time Relationships for Exposure to Ammonia (adapted from [8-10])

<u>Exposure Level (ppm)</u>	<u>Exposure Time</u>	<u>Symptoms</u>
1-50	Indefinite	Odour threshold
40		Eye irritation in some exposed individuals
100	minutes to indefinite	Eye irritation pronounced; acclimation possible
200		Irritation of mucous membranes; bearable
400		Severe irritation of throat, nose, and upper respiratory tract
700	1/2 hour	Severe eye irritation and of upper respiratory tract
500-1000		Strong irritation of upper respiratory tract
1700	1/2 hour	Serious coughing; bronchospasms
2000-2500	1/2 to 1 hour	Dangerous toxicity; fatal to some individuals
5000	5-10 min or less	Serious edema; dyspnea; asphyxia; enhanced risk of death

2.2 MODE OF ACTION

2.2.1 Respiratory System

When individuals or test animals are exposed to high levels of ammonia in air, the predominant effects are of a topical or local nature -- the gas acts as a primary irritant to exposed surfaces. Systemic changes undoubtedly also occur, often as a secondary physiological reaction to the acute effects at respiratory surfaces. In addition, systemic changes directly attributable to absorbed ammonia have been investigated more commonly in studies of long-term effects resulting from exposure to low levels of ammonia. The modes of action in the two categories of systemic effects differ significantly.

2.2.1.1 Uptake: The high solubility of ammonia gas in water allows it to be absorbed at moist surfaces, in particular the eyes and respiratory system. The ready absorption of ammonia in the moist lining of the upper respiratory tract may serve to protect the lower tract structures from serious damage, in particular sensitive bronchioles and alveoli. Such a "naso-bucco-pharyngeal filter" for ammonia has been demonstrated by Boyd *et al.* in rabbits [10] and it has been shown that ammonia from inhaled air can be retained very efficiently in the human nose [11], at least at the low concentration (72 ppm) tested in human volunteers. The low-level exposures in humans were carried out for a maximum of two minutes, a time probably not long enough to achieve a plateau for absorption of ammonia into the respiratory system lining. Nonetheless, it was demonstrated that 91 to 93 percent can be retained in the lung and 63 to 83 percent in the nasal membranes. Silverman *et al.* [12] evaluated uptake using 500 ppm levels, breathed for 30 minutes. Ammonia retention started out maximally and then decreased so that by about 10 to 20 minutes a plateau was achieved, at which point only 20 percent of the inhaled ammonia continued to be retained. Presumably this entered the internal body system to be distributed and metabolized at a relatively constant rate. After cessation of exposure, no further ammonia was released through exhalation. Although there is some

question as to the actual levels of ammonia which circulate in the blood stream during and after exposure, there is no question that ammonia is absorbed [13-16].

At high exposures, one may speculate that the filtering capacity of the upper respiratory tract is exceeded when its moisture layer approaches a concentration of ammonia in water close to 28 percent (concentrated ammonium hydroxide) allowing a greater amount of ammonia gas to enter the lower respiratory tract. An extensive study using bats demonstrated very well the consequence of loss of a nasopharyngeal filter [17]. Bats, exposed to ammonia concentrations up to 3000 ppm, tended to breathe through the nose and survived this concentration without major debilitation even at exposures as long as nine hours. During exposure to 3500 ppm, however, the bats changed from nasal to mouth breathing and died within four hours of exposure. It can be suggested that blockage of the nasal passages was caused by a swelling of epithelial tissues, promoted by the irritant action of ammonia. A switch to mouth breathing would be a natural consequence of this blockage, along with loss of the naso-bucco-pharyngeal filter.

2.2.1.2 Exposure effects: There is little question that the upper respiratory tract can be a target site for damaging effects. The irritant action of ammonia at high concentrations has been demonstrated by the appearance of lesions in the mouth, nose and throat in individuals exposed accidentally [18-22]. An additional and more life-threatening complication is the tendency for laryngospasm or bronchospasm, responses that have been observed even after exposures of very short duration [21-25].

The effects of acute exposure on the lower respiratory tract (i.e., the respiratory surfaces and supportive tissue components) can be severe. Accidental exposure has resulted in early symptoms, including massive edema and extravasation resulting from destruction of mucous and alveolar membranes, as well as blood vessels. Symptoms associated with the lung damage were chest pain, shortness of breath, and a cough producing bloody, frothy sputum [21,22,26].

Some types of lung damage are commonly found to be delayed, that is, beyond the relatively short-term effects associated with the period during or immediately after exposure. In general, edema may continue, a sloughing of epithelial layers is possible, and gas exchange becomes more difficult. The lungs can become fluid filled. An apparent recovery period may be followed by a marked susceptibility to pulmonary infection and deaths from pneumonia have been recorded [18,25,27,28].

Less is known about a direct systemic response to high levels of ammonia in circulation. But it is recognized that ammonia (i.e., as the ammonium ion) at levels greater than 8 to 11 mg/100 mL in rabbits is debilitating and can cause death [15].

Localized effects on the respiratory tract surface are correlated with a number of changes in respiratory and cardiovascular physiology which are at least partly compensatory in function. Short-term low-level exposure in humans tends to have little effect on pulmonary physiology. For instance, exposure to 140 ppm ammonia for up to two hours caused no changes in vital capacity or in forced-inspiratory or -expiratory volumes but was considered to be irritating [29]. A study of exercise ventilation was carried out on military volunteers exposed to ammonia at 73 to 499 ppm [30]. At the lower doses, there was a small reduction in ventilation minute volume due to a decrease in respiratory frequency; at the higher doses a similarly small reduction (average 6%) in ventilation minute volume was due to a reduction in tidal volume accompanied by tachypnoea. A similar experimental study with humans examined changes in respiratory volumes in resting individuals, exposed for 30 minutes to 500 ppm of ammonia [12]. Again, there were changes, but these were somewhat different from the exercise test. Respiratory rate increased slightly (i.e., tachypnoea), but respiratory (ventilation) minute volume doubled during the exposure period. None of the exposures in the described experiments resulted in any residual effects.

The effects of short-term exposure to ammonia on respiratory volumes and frequency have been verified in laboratory animals. When rabbits were exposed for up to nine hours to 50 or 100 ppm of ammonia, a decrease in respiration rate accompanied by an increase in depth of respiration was observed [31]. The influence of ammonia at such low levels of exposure appears to be directly on the receptors in respiratory epithelia. It has been demonstrated, for example, in cats and in rabbits that irritant (or deflation) receptors, as well as pulmonary stretch receptors, are stimulated by ammonia [32,33]. In effect, the relative balance of stimulation will influence tachypnoea, bronchoconstriction, and the volumes and pattern of breathing. The field has been extensively investigated, but a discussion of it is beyond the scope of this report.

2.2.2 Nervous System

The mammalian body possesses an effective mechanism for eliminating ammonia in circulation (as the ammonium ion) by metabolic conversion in the liver to urea, which is then excreted in the urine [34]. A swamping of this system by an excess of ammonia in circulation, as may be expected after acute inhalation of high levels of ammonia gas, is likely to disturb physiological and biochemical functions at a number of sites. Neurological symptoms, for instance, may be caused by one or several mechanisms of toxic systemic action of ammonia: a decrease of acetylcholine levels in the brain [35]; an increase in monoamine-oxidase activity in the cerebral cortex and a decrease in the cerebellum and brain stem [36]; interruption of cellular respiration [37]; and a decrease of both glycogen and glucose levels in the brain [38]. Although incompletely understood, ammonium ions in excess cause irregularities in normal cation movement across cell membranes -- a net efflux of potassium ions from cells is a feature of ammonia treatment [39] -- and this can be a contributing factor to neurological symptoms. Because ammonia is eliminated quite rapidly from the body, such effects are more likely during the exposure periods than during the recovery period.

While it has been possible to characterize a number of biochemical changes of neurological consequence, it would be difficult to separate these from the effects of respiratory impairment as causes for convulsions and unconsciousness resulting from acute exposure to ammonia. Both, however, would be contributory to an assessment of death risk.

2.2.3 Eyes and Skin

Damage to eyes and skin following an acute exposure incident is much less likely to result in death than the respiratory effects. The mode of action on these organs can be as a primary irritant and by freezing.

On moist surfaces such as the eyes, massive exposure to ammonia gas in the air has caused pronounced lesions and even permanent blindness in humans [21,23,24,28]. The effects resemble burns caused by exposure of the eyes and face to concentrated aqueous ammonia (ammonium hydroxide) [40,41]. Skin damage can result from acute exposure to ammonia [20,22,23,27,28], both by direct irritant action of ammonia gas and by a freezing effect from contact with liquid ammonia.

2.3 CHRONIC EXPOSURE

The effects of chronic or sequential exposures have been extensively investigated and reviewed, mainly in the context of evaluation of occupational- and environmental-health risks [42,43]. Discussion of these in the context of this report has little merit since no substantive contributions can be made from such studies to an interpretation of health effects resulting from short-term high-level exposures. Ammonia does not appear to be retained in the body so that no long-lasting systemic intoxication can be postulated.

In general, chronic exposure effects include eye irritation, nasal and pulmonary irritation and damage, and biochemical changes. The relationships are dependent on both level and duration of exposure, as well as on the species tested. Humans seem able to tolerate continuous exposure to 100 ppm without negative health effects -- acclimation appears to be possible up to 100 ppm with respect to both comfort tolerance and irritant effects [44]. Exposures above 100 ppm were found to be uncomfortable, leading to the conclusion that no permanent injury is likely to occur from exposure to ammonia throughout a working lifetime at levels below those that cause intolerable (i.e., unacceptably uncomfortable) acute effects [45].

2.4 EXPOSURE EFFECTS IN HUMANS

2.4.1 Accidental Exposures

The effects of acute exposure to high levels of ammonia are demonstrated in reports of accidental exposure cases. Twenty-six reports documenting the immediate and longer-lasting consequences of an even greater number of exposure incidents have been evaluated and are summarized in Table 2-2. The circumstances of accidents are diverse, but usually involve a sudden or explosive release of ammonia from a contained system. It is undoubtedly the response to the accidental and emergency nature of the exposure incidents which have been prime reasons for a lack of data on atmospheric concentrations. Attempts have been made to estimate the exposure levels beyond subjective evaluations of "low" to "high". Mulder and Van der Zalm described an acute case resulting from exposure to ammonia released to the atmosphere when a tank of ammonium hydroxide overflowed [51]. Based on the ammonium hydroxide concentration and weather conditions, the ammonia concentration was estimated as 10,000 ppm. The exposure, estimated to be for a few minutes, resulted in death six hours after the accident.*

* A recent (unpublished) review of this incident, using information from internal documents of the company involved in the accident, estimated that the victim was exposed to 20,000 ppm ammonia for half an hour, rather than 10,000 ppm for a few minutes [76].

TABLE 2-2 Effects of Accidental Exposure to Ammonia

Reference	Occupation	Circumstance	Exposure Data		Fatality Incidence/ No. Cases	Time	Uncon- sciousness	Remarks
			Level	Duration				
[18]	Industry	refrigeration explosion	low to high	<1 h	1/6	1 mo	ND	Body and facial skin burns; in case of fatality showed lung congestion, hemorrhage, acute bronchopneumonia, edema, acute laryngotracheitis.
[26]	NA	refrigeration condensor leak	ND	ND				75 cases hospitalized, 47 considered significant.
					0/9 in category	NA	ND	Mild cases (9/47) - few effects.
					6/27 in category	6 h and 2-3 d	ND	Moderate cases (27/47) - pulmonary edema; bronchopneumonia.
					7/11 in category	< 2 d	ND	Severe cases (11/47) - pulmonary effects - edema, pneumonia, etc.
[46]	refrigeration workers	cooling system rupture	high	minutes	3/4	5 min to 5 h	one immediate	Exposure in a closed room; one worker became unconscious and died in 5 min; 2 others suffered from respiratory distress and died in 2 h and 5 h; the survivor suffered for months from bronchial irritation and hemoptysis.
[48]	ND	ND	high	ND	0/1	NA	ND	Examined 5 mo to 3 y from accident; at 5 mo showed bronchopneumonia; became chronic parenchymatous fibrosis and emphysema.
[49]	Industry	working accident	ND	ND	0/1	NA	ND	Follow-up study (1-17 mo) of 1 case; progressive deterioration of maximal respiratory capacity, of vital capacity; diffusion capacity reduced.

[50]	factory workers	refrigeration tubing rupture in freezer unit	high	seconds to min?	4/6	within min. 5 d	ND	2 workers escaped immediately and were not greatly affected; 4 others not able to get out - 2 died during exposure; autopsy showed massive burns and hemorrhage in glottis and larynx and trachea, also extensive pulmonary edema; 2 died in 5 d in hospital, autopsy showed purulent lesions of larynx and trachea, edematous pulmonary parenchyma, bronchopneumonia.
[23]	agriculture	fertilizer use	high	ND	0/4	NA	ND	All 4 cases required tracheostomies; facial and other skin burns extensive; respiratory tract lesions; eye injuries (permanent blindness in one); dyspnea.
[51,76]	Industry	overflow of tank of ammonium hydroxide	est. 10,000 or 20,000 ppm	few minutes or 172 hr	1/1	6 h	ND	Victim continued to work after accident; 3 h post-exposure had red and swollen face, conjunctivitis, raw mouth and throat, labored breathing; condition worsened rapidly; died of heart failure 6 h post accident; marked inflammation of respiratory tract, denudation of tracheal epithelium; no pulmonary edema.
[52]	truck driver	ruptured container	ND	ND	0/1	NA	ND	Immediate dyspnea and lacrimation; respiratory recovery in 1 d; recurrent severe gastritis over several months.
[40]	bank teller	aqueous ammonia thrown into face during robbery	ND	ND	0/1	NA	ND	Marked edema of nasopharynx and glottis within several h; chemical pneumonia for 1 w; burns of eyes and ocular membranes with serious permanent impairment in 1 eye.
[53]	NA	rail accident with tank car rupture	ND	long	8/78	ND	ND	70 exposed people were injured; heavy fog held vapors close to ground for a long time.
[54]	worker	refrigeration tubing rupture	ND	~1 min	0/1	NA	ND	Immediate dyspnea and burning eyes; severe respiratory distress 20 d later; 1 y later still suffered from chronic bronchitis and obstructive lung disease.
[41,55]	various	aqueous ammonia thrown into eyes of robbery victims	ND	ND	0/3	NA	ND	Eye damage: edema of conjunctivitis, corneal damage, loss of iris reflex, high intraocular pressure, glaucoma, blindness.

TABLE 2-2 Continued

Reference	Occupation	Circumstance	Exposure Data		Fatality Incidence/ No. Cases	Unconsciousness	Remarks
			Level	Duration			
[19]	ND	various	ND	ND	NA	NA	Follow-up study of 8 cases; 2 with moderate acute phase damage (i.e., burns to upper respiratory tract and no pulmonary involvement) had minor to medium severity pulmonary impairment 1-2 y after exposure. 2 victims with medium acute phase damage had restrictive ventilation problems of medium to major severity 1 to 1.5 y later; gas exchange impaired. 4 victims with major acute phase damage (i.e., parenchymatous effect dominant, acute pulmonary edema) all showed significant latent pulmonary impairment 1-4 y following exposure; 1 showed gradual deterioration in pulmonary function over 4 y; generally damage was of a restrictive and/or obstructive nature with impaired gas exchange.
[20]	agriculture	fertilizer equipment accidents; transfer operations	medium to high	>1< few min	0/4	ND	Skin burns following contact; delayed pulmonary obstruction and dyspnea; eye lesions; no permanent pulmonary damage.
[21]	mechanic	refrigeration accident	high	5 min	0/1	yes <5 min	Tracheostomy required; respiratory impairment; coma; skin burns; diminished reflex response; no permanent eye damage; recovered.
[56]	NA	tank-car derailment	ND ND	30 min 90 min	0/2	NA	Respiratory distress; bronchiectasis; latent effect.

[21]	Industry	various accidents with liquid NH_3	light	>1< few min	0/1	NA	ND	Blepharospasm; bronchospasm (1 case).
			heavy	"	0/4	NA	3/4 within min	Dyspnea; chest pain; blepharospasm; burns on skin and of oral/respiratory membranes; bronchospasm (4 cases); gas transfer impaired for up to 3 y.
			very heavy	"	1/2	Immed.	ND	Tightness of chest; blepharospasm; bloody sputum (2 cases).
[27]	fertilizer manufacture?	tank explosion	ND	ND	4/4	3 Immed; 1 2 mo	ND	Skin burns; respiratory distress; pulmonary edema; 2 mo to secondary nodocardial bronchopneumonia; bronchiectasis; fibrous obliteration of small airways - obstructive lung disease.
[57]	NA	rail accident, 2 tank cars ruptured; massive release	ND	3 h	2/4	ND	ND	A man and wife died, their 2 children were injured and required hospital care; ND on how many people affected besides these 4.
[22]	NA	tank truck explosion	low to high	<1/2 h	7/60	ND	yes	Skin, eye and respiratory surfaces burned or irritated; high exposures could be fatal due to upper airway obstruction; fatalities avoided in some cases by tracheostomies; lower level exposures of longer duration resulted in long-term pulmonary effects.
[25]	fishing industry	refrigeration accidents	ND	sec to min	0/14	NA	ND	(14 cases) mild to moderate respiratory functional impairment; coughing; chest pain; dyspnea; eye effects; corneal burns; airway obstruction requiring treatment in 5? cases (tracheostomy in 1).
[28]	NA	tanker truck accident	heavy	20 min	1/1	35 d	semi-consc. in 3 h	Skin burns; cystic bronchiectasis; pneumonia; loss of bronchial cartilage; burns of eyes.

NA = not applicable

ND = no data or not found

The initial targets of ammonia toxicity are the nasal membranes and linings of the throat and mouth; eyes and facial skin are also affected [18,20-25,28,40,41,50,51,55]. The mucosal lining of the upper respiratory tract may become swollen and edematous, with sensations of burning and restrictions to nasal airflow. In severe cases, blistering and sloughing of the epidermal layers may occur. Also, in severe cases the trachea may be affected, showing irritation, edema, sloughing, and bleeding.

Spasmic contraction of the laryngeal area and of the bronchial pathways may occur, most probably after or during fairly high levels of exposure [18,21,22,24,25]. As discussed previously, this is a reflex response due to the irritant action of ammonia on mucosal receptors in these areas. While such laryngo- and bronchospasms may be considered protection of the lower respiratory tract in that they restrict continuing exposure to the airborne irritant, they can of course be fatal in themselves if airflow is extensively restricted. The exponential relationship between tracheal diameter and amount of air flow makes even small amounts of constriction significant. There was an apparent requirement in a large number of severe cases for emergency tracheostomies in order to counteract a tendency for acute asphyxiation in the victims.

A third level of target action is the lower respiratory tract, with pulmonary damage characteristic in the more debilitated victims (i.e., those presumably exposed to higher levels of ammonia or for longer durations) [18,19,22,26-28,48-50,54]. Subjectively, this damage produced difficulties in breathing marked by restriction in ventilation, dyspnea, and chest pain. A bloody, frothy mucous was seen occasionally. Clinically this acute phase was defined as massive pulmonary edema and hemorrhage. The pulmonary damage could be so severe that death resulted, although this was not necessarily always the case. Broncho- or pulmonary pneumonia was a not uncommon consequence to damage of lower respiratory tract membranes. Pneumonia tended to develop within one week to two months following exposure and was the cause of death in some victims [26-28,50].

Overall, the range of severity of acute responses to accidental exposures to ammonia is wide. Short-term, estimated low-level exposures tended to cause localized irritations with no serious impairment and did not require extensive treatments. Medium-level exposures of short to medium duration (i.e., up to a few minutes) have caused a spectrum of responses, which invariably required treatment. Recovery tended to be rapid, although the tendency for spasmic contraction of the upper airways was occasionally a complicating and potentially lethal factor. Exposures estimated to be high caused serious immediate and longer-term effects, irrespective of whether the initial exposure was of short or longer duration. An overloading of the nasopharyngeal filter is probable and pulmonary damage could be extreme. The best evaluations for dose-response relationships come from reports by Walton [21], Close et al. [22], and Calpin [26], and these are summarized in Table 2-2. The overall fatality index, taking into account all cases of accidental exposure shown in Table 2-2, was 44 deaths among 251 exposed individuals, or 18 percent. The majority of these cases were men, although women also died from ammonia in a few instances [54,56].

A final consideration with respect to toxicity of ammonia in humans is that there may be a long-lasting, even permanent respiratory impairment. This impairment was shown particularly in individuals who demonstrated effects of an acute nature in the lower respiratory tract during and soon after the exposure incident [26-28,50]. Initial pulmonary damage in these cases could be so extensive that complete recovery would not occur; respiratory functions would remain impaired, with restricted air flow through air passages and decreased gas exchange at respiratory surfaces. These sequelae were due to fibrous obliteration of small airways (an emphysematous condition) and chronic bronchitis.

2.4.2 Experimental Exposures

The results of experimental exposure studies in humans do not greatly aid the interpretation and prediction of acute effects from accidental releases of ammonia and, in particular, are of little value for prognostication of lethality: ammonia is so uncomfortably irritating at levels above 200 to 300 ppm that voluntary exposure would probably require an exceptional effort of will. As may be seen from the summary of experimental exposure data (Table 2-3), the maximum exposure tested was 530 to 560 ppm for four hours -- a remarkable feat by the individual concerned. The utility of these studies lies in verifying target tissues susceptible to the irritating effect of ammonia. Also, a number of physiological changes could thus be examined which would have been swamped by the severe acute effects in a high-level exposure. It does not seem that the effects of even 500 ppm of experimental exposure could be considered life-threatening.

The experimental data indicate an odour threshold for some individuals as low as 0.65 ppm [58], although an entire test population could detect ammonia between 30-50 ppm [59-61]. Irritation of the eyes and upper respiratory tract seems to have a threshold of 50 to 70 ppm if administered for up to 10 minutes [60,61]. Changes in respiratory functions appear to require levels of ammonia greater than 100 to 200 ppm [6]. Ammonia is well absorbed, showing up in the blood at exposures of 19 ppm, although not at 4 ppm [16]. This suggests that normal metabolic conversion and excretion mechanisms (i.e., as urea in urine) are exceeded by eight hours of exposure at 19 ppm. Ammonia is voided rapidly, however, at the cessation of exposure -- even at the highest levels tested [12].

TABLE 2-3 Effects of Single Experimental Exposures to Ammonia - Human Studies

Reference	Concentration (ppm)	Duration	No. subjects male or female	Results
[13]	530-560	4 h	1 M	Increase in non-protein nitrogen in plasma (NH_3 -derived) and decrease in blood pressure in first 35 min.
[12]	500	15-30 min	7 M	Respiratory minute volume increased 50 to 250% above control; hyperventilation after exposure; irritation of nose and throat; lacrimation; at equilibrium about 20% of inhaled ammonia absorbed; rapid return to normal of all responses after end of exposure.
[6]	73-499	35-60 min	18 M	Changes in volumetric respiratory parameters and in rate of breathing, markedly so at higher levels.
[2]	200, 300 and 330	20 and 30 min	3 M	Trials subjective; nasal irritation and congestion, sneezing; deep inhalation due to sneezing caused tracheal pain; eye irritation; salivation; nausea; headache; some accommodation after 5 min of exposure; symptoms alleviated after a short while following exposure.
[29]	50-140	to 2 h	13 M, 3 F	No effect on volumetric respiratory functions; eye, nose and throat irritation; 140 ppm not tolerated for 2 h; psychological factors play role in reaction of individuals.
[61]	32, 50, 72, 134	5 min	8 M, 2 F	At 32 ppm, 9/10 detected odour; no irritation at 50 ppm, all detected odour; at 72 ppm, low incidence of minor eye and nose irritation; at 134 ppm, majority reported lacrimation and irritation of eyes, nose, mouth, or throat; one reported slight chest irritation.
[60]	30, 50	10 min	6	At low dose, half of subjects noted minor eye and nose irritation and could smell odour. At 50 ppm, 4/6 noted moderate eye and nasal irritation; all noted odour.
[59]	46.8	ND	4	All four subjects recognized the odour.
[16]	4.35, 18.9	8 h	6 M	At 18.9 ppm, increase in blood and urine NH_3 , and urine urea levels; tendency for decrease in coefficient for oxygen consumption; no latent changes. No changes at 4.35 ppm.
[10]	0.65-1.45	ND	22	Threshold of perception of ammonia as low as 0.65 ppm in a few individuals.

ND = no data or not found

2.5 EXPERIMENTAL STUDIES IN ANIMALS

The focus of this part of our report is on results of acute single exposure studies using laboratory animals, with particular emphasis on lethality data. Many long-term, repeated-dose or chronic studies have also been carried out with a variety of laboratory species. In general these chronic studies model responses identified in humans exposed for longer time periods to low levels of ammonia. Recent reviews of these findings are found in NIOSH [42] and Carson *et al.* [43].

Experimental studies of acute effects have relied mainly on mice, rats, rabbits, and cats to demonstrate pathological effects of exposure to ammonia. A summary of the various findings has been made by the Deutsche Forschungsgemeinschaft in 1972 [62] in derivation of their MAK-values. Table 2-4 has been drawn from Kimmerle [8] who cites this summary. Deaths appear to occur at concentrations greater than 3000 ppm if exposure occurs for two or more hours. It is only at very high levels, above 10,000 ppm, that deaths seem to occur after only a few minutes of exposure to ammonia. A more detailed evaluation of the animal data for acute effects of ammonia is presented in Table 2-5 and is discussed in the following sections. (The citations in the table represent the majority of recent publications assessing single exposure toxicity in laboratory species.)*

* A small number of much older studies have been described by Lehmann [2] in his treatise on the toxicity of noxious gases in the working environment. But these older studies suffer from a lack of quantification of both exposure concentration and effects and are therefore not detailed in this report. Even from these studies, however, it was evident that high concentrations caused breathing dysfunction, upper respiratory tract damage, lung damage, and sometimes death after a few minutes of exposure.

TABLE 2-4 Summary of Inhalation Toxicity of Ammonia in Animals*

<u>Species</u>	<u>Duration of Exposure</u>	<u>Effect</u>	<u>Concentration in Air (ppm)</u>
Various	4 h	irritation	480-570
Various	7 h	strong irritation	800-1,070
Various	5-7 h	dyspnea, tracheitis	820-1,430
Rat	4 h	no deaths	2,000
Mouse	2 h	minimum lethal concentration	3,420
Rat	4 h	fatal	4,000
Mouse	2 h	LC ₅₀	4,760
Rat	2 h	LC ₅₀	10,930
Various	few minutes	immediately fatal	20,000-30,000

* from Deutsche Forschungsgemeinschaft [62], as cited in Kimmeler [8].

2.5.1 The Mouse

In recent years, a number of reasonably rigorous studies into the lethal effects of ammonia have been carried out using mice. Behaviourally, mice respond to acute exposures initially by closing of eyes, dyspnea or gasping, and terminally with tremors, ataxia, coma, and tonic seizures ending in death [65,68]. Respiratory tract irritation is evident both in the upper pathways and in the lungs [64,65,68]. High levels of exposure (e.g., 4,000 ppm or greater) for up to one hour result in lung congestion and hemorrhage during exposure. A 1,000 ppm exposure administered for 16 hours had similar effects. A two-phase response is suggested, in that at high exposure levels, above 10,000 ppm, death occurred during the (less than one-hour) exposure period [65-67], while exposure up to about 5,000 ppm for one hour resulted in significant mortality within several days post-exposure [68]. It is probable that the second phase is caused by gradual degenerative changes in the lungs and a greater susceptibility of the damaged tissue to infection (pneumonia). Aside from eye irritation (an effect that is observable at comparatively low concentrations), other organ systems which were affected by ammonia were the gastrointestinal tract, kidneys, and liver [64,68]. There is experimental proof that female mice are less susceptible to ammonia effects than male mice. The data of Stupfel *et al.*, for example, suggest a difference in susceptibilities by a factor of about two after exposures of 12,000 ppm for 20 minutes; a smaller difference is observed in longer exposures [66]. No mechanistic explanation of the difference, however, was provided by these authors.

TABLE 2-5 Effects of Acute Experimental Exposure to Ammonia

Reference	Species	Exposure Data		Fatality		Unconsciousness	Remarks											
		Level (ppm)	Duration	Incidence	Time													
[64]	mouse, rat	16-1000	1-960 min	50%	>16 h	ND	At high levels distended hemorrhagic lungs; distended heart; congested liver and kidneys; distended gastrointestinal tract; LT_{50} greater than 16 h at 1000 ppm.											
[10]	mouse	5,800	30 min	32%	48 h	ND	Deaths in the course of 48 h. (Concentration is estimated av. over exposure period; starting concentration 11,600 ppm.)											
[65]	mouse	8,845-13,050	10 min	25-80%	--	5 min	10 min LC_{50} calculated as 10,237 ppm; initially great excitement; eyes closed immediately; gasping within 1 min; respiratory tract irritation; 93% of deaths during exposure; latent deaths after 7 to 10 days.											
[66]	mouse	12,000 11,000	20 min 40 min	71% 84%	20 min 40 min	ND	Male mice more susceptible: <table><tr><th colspan="2">Fatality Incidence</th></tr><tr><th>Exposure</th><th>Male</th><th>Female</th></tr><tr><td>12,000 for 20 min</td><td>92%</td><td>48%</td></tr><tr><td>11,000 for 40 min</td><td>94%</td><td>74%</td></tr></table> No deaths occurred in 48 h post-exposure period.	Fatality Incidence		Exposure	Male	Female	12,000 for 20 min	92%	48%	11,000 for 40 min	94%	74%
Fatality Incidence																		
Exposure	Male	Female																
12,000 for 20 min	92%	48%																
11,000 for 40 min	94%	74%																
[67]	mouse	28,571 26,190 23,810 (range 7,143 to 28,571)	30 min	100% 100% 75%	4.7 min 4.4 min 5.3 min	ND	LT_{50} values mathematically derived by the author as average times to death of 4 mice tested (for results of an alternative analysis see Table 3.2); at lower concentrations not all 4 mice died in 30 min test period; a latent death at 19,048 ppm; no deaths at 14,286 ppm and less.											
[68]	mouse	0-4860	1 h	yes	15-20 min at high conc., longer at low conc.	yes	Signs of acute toxicity were tremors, ataxia, convulsions, coma, and progressing to toxic seizure and death; dyspnea; weight loss; elevated liver weight; lung congestion and hemorrhage during exposure; latent pneumonitis.											
[69]	mouse	4,837	ND	50%	1 h	ND	Summary review.											

TABLE 2-5 Continued

Reference	Species	Exposure Data		Fatality		Unconsciousness	Remarks															
		Level (ppm)	Duration	Incidence	Time																	
[2]	rat	480-31,300	32 min to 5.5 h	1/1 (1/7 total)	32 min	no	Tests carried out on individual animals; <u>fatality only at highest exposure level, with dyspnea and lung hemorrhage</u> ; minor symptoms at other levels, some suggestion of narcotizing effect, total recovery.															
[70]	rat	2000	4 h	33%, 50% or 67%	4 h	ND	14 d post-exposure observation period; ammonia belonged to group of gases which caused 2/6, 3/6, or 4/6 animals to die as a result of exposure to 2,000 ppm for 4 h.															
[71]	rat	14,312-54,839 40,300* 28,595* 20,300* 16,600*	10-60 min	50% 50% 50% 50%	10 min 20 min 40 min 60 min	ND	LC ₅₀ evaluation study; LC ₅₀ s calculated from experimental data; males more susceptible than females: <table><tr><th>time</th><th>male LC₅₀</th><th>female LC₅₀</th></tr><tr><td>10 min</td><td>37,380</td><td>45,300</td></tr><tr><td>20 min</td><td>25,710</td><td>32,900</td></tr><tr><td>40 min</td><td>17,670</td><td>23,910</td></tr><tr><td>60 min</td><td>14,200</td><td>19,850</td></tr></table>	time	male LC ₅₀	female LC ₅₀	10 min	37,380	45,300	20 min	25,710	32,900	40 min	17,670	23,910	60 min	14,200	19,850
time	male LC ₅₀	female LC ₅₀																				
10 min	37,380	45,300																				
20 min	25,710	32,900																				
40 min	17,670	23,910																				
60 min	14,200	19,850																				
[2]	guinea pig	480-4600	410 h	0/4 total	NA	no	Low levels caused slight irritation and decrease in respiration; higher levels (2580-4600 ppm) caused moderate symptoms of irritation, no eye opacities; post-mortem showed hyperemic and slightly edematous lung.															
[2]	rabbit	800-4600	2.5-10 h	0/5 total	NA	no	Slight symptoms of irritation below 2000 ppm, decreased respiration rate; above 2000 ppm more irritating, some eye irritation and cloudy corneas; above 2600 ppm, further decrease in respiration rate, hemorrhagic trachea, with destruction of epithelial lining, ecchymoses under skin, lung chambers collapsed.															
[72]	rabbit	NA	NA	NA	NA	NA	Temporary but major rise in intraocular tension when ammonia solution applied.															

[31]	rabbit	50 and 100	3 h					Respiration rate decreased approx. 1/3; depth of respiration increased; HUN and blood CO ₂ increased; no tissue pathology.
[73]	rabbit	1,000-20,000	1 h	ND	ND	ND	ND	(Post-exposure period 2 h); lowest effective dose, 6000 ppm; lethality from 6000 ppm; higher exposure levels, shorter response time; bradycardia early effect; deaths follow exposure if exposed greater than 7400 ppm. Experiment used artificial respiration of curarized animals and, apparently, only one animal at each exposure level.
[10]	rabbit, cat	~ 5,000	1 h	~ 50%	1 h	~ 1 h		Output of respiratory tract fluid increased, as did respiratory iron content; blood Hb increased; histological damage prevalent. (Concentration is estimated average over the exposure period; starting concentration was at least twice as high.)
[69]	rabbit, cat	10,066	ND	50%	1 h	ND		Summary report.
[2]	cat	480-1,440	4-7 h	1/4 total	NA	no		Low levels caused initial signs of irritation, salivation and decreased respiration; 800-1100 ppm resulted in irritation, some quieting effect, persistent salivation, and decreased respiration; 1100-1400 ppm resulted in dyspnea, evidence of pain, marked decrease in respiration, recovery after exposure; high dose trauma including bleeding of nasal membranes, suppurative tracheitis, hyperemic lungs with ecchymoses.
[74]	cat	1,000	10 min	0/20	NA	ND		(35 d follow-up); at least 2-phase response; necrosis and sloughing of respiratory mucosa by 5 d; bronchoconstriction reflexes can protect lungs, minimize functional changes; latent bronchitis and bronchopneumonia; increased resistance and decreased dynamic compliance was a latent response.

NA = not applicable

ND = no data or not found

* These LC₅₀ values refer to the mixed female/male population.

2.5.2 The Rat

A survey of the data on rats demonstrates this species is less susceptible to ammonia than mice. Fatalities tended to occur at higher exposure levels (for example at 32 minutes for 31,300 ppm [2] or at 10 minutes for 40,300 ppm [71]) than those required for mice. As with mice, the female rat is clearly more resistant to ammonia than her male counterpart; LC₅₀ values for females were found to average some 30 percent higher than those for males [71]. This difference in LC₅₀ values appears to decrease as exposure time increases -- being about 40 percent at 10 minutes of exposure but only about 20 percent at 60 minutes. Behaviourally, rats show the usual symptoms when exposed to ammonia -- dyspnea, upper respiratory tract irritation, and some narcotizing effect. There are no data on latent mortality resulting from acute exposures.

2.5.3 The Guinea Pig

Guinea pigs have been little studied with respect to ammonia inhalation. This lack of data is, perhaps, unfortunate because guinea pigs may be somewhat more sensitive than rats or mice. They show moderate symptoms of irritation and lung damage after several hours of exposure levels as low as 2580 and 4600 ppm [2]. There were no mortalities during these exposures; follow-up studies, however, were not carried out.

2.5.4 The Rabbit

Rabbits are also fairly sensitive to ammonia inhalation [2,10,31,72,73] and exposures as low as 100 ppm given for three hours [31] have caused changes in breathing patterns. A few hours of exposure above 2000 ppm resulted in decreased respiration rates, eye irritation, and cloudy corneas [2]. Such levels also carried lesions of epithelial linings of the upper respiratory tract and lung damage. Histological damage was prevalent with one hour of exposure to an average of 10,000 ppm [10]. In an experiment

using artificially-ventilated, curarized rabbits (no laryngospasm possible in this case), deaths occurred from ammonia inhalation at levels greater than 6,000 ppm [73]. Other physiological changes included bradycardia, encephalographic alterations, and blood gas changes.

2.5.5 The Cat

Cats also appear to be sensitive to low levels of ammonia. Irritation was evident at low levels (480 ppm), along with decreased respiration [2]. Tests at 1100 to 1440 ppm for several hours resulted in a marked response, described as dyspnea, evidence of pain, upper respiratory tract lesions, and lung damage. The cat may serve as a good human model, in that it is both sensitive to ammonia exposure and has been shown to demonstrate an at least two-phased response. It was a point of interest to Dodd and Gross [74] that a domestic cat exposed to ammonia after a tank truck accident in 1976 was affected similarly to human victims exposed at the time, especially with regard to longer-term chronic effects resulting from the acute exposure. These were latent bronchitis, bronchopneumonia, emphysema, and fibrosis, all of which affected respiratory functions in a progressive manner. There are few data concerning lethality, although a one-hour LC₅₀ of 10,066 ppm has been suggested [69]. But, for the cat, experimental verification of lethality limits is poor.

2.6 SECTORS OF POPULATION AT GREATER RISK

Within any population, a certain proportion will exhibit greater susceptibility to the effects of a toxicant than the majority. The human and animal data discussed in the preceding sections provide evidence for a number of factors which will have bearing upon the severity of response an individual may experience when exposed to high concentrations of ammonia.

Although there has been no evaluation of the contribution of health status to the expression of ammonia toxicity, it is reasonable to assume that an individual who is already suffering from impairment in respiratory function would be more readily affected by ammonia toxicity. Chronic bronchitis, chronic rhinitis, pneumonitis and edema, and emphysema are all conditions which could be expected to increase the severity of effects resulting from exposure to ammonia. It should be remarked that individuals with laryngeal and other upper-respiratory-tract irritation (such as caused by the common cold, for instance) may also be more susceptible to the onset of laryngo- and tracheospasm.

There is experimental evidence that the sex of the individual may influence the toxicity of ammonia in that individual. Both female mice [66] and rats [71] were found to be more resistant to death from acute exposures to high levels of ammonia than their male counterparts. A factorial difference of as much as three with respect to concentration is indicated for the rodents in these studies. No such differential can be established from the human data base, although the animal data may cause one to speculate that men are more susceptible to ammonia than women. (More men have died as a result of ammonia inhalation, but this is a consequence of a greater incidence of exposure because males comprised the predominant proportion of the work force at risk.) More qualitatively, both men and women have been severely affected by and died from ammonia inhalation.

The age of the exposed individual may also influence the expression of ammonia toxicity. In general terms, older people show a reduced respiratory capacity as compared to younger adults and thus may be more readily impaired. (Functions such as vital capacity and compliance are known to decrease normally with age.) There are no data, however, to evaluate this differential with respect to ammonia toxicity. Children are usually assumed to be more sensitive in acute exposures to atmospheric toxicants. They have a lower ventilatory volume but a higher ventilatory rate than adults (newborn babies -- 500 mL/min, 33 breaths/min; children -- 20 to 25

breaths/min; adults -- 6 L/min, 12 breaths/min). Where the balance of these two factors falls in relation to susceptibility cannot be easily assessed. If one wished to speculate, it could be supposed that young children would be more susceptible to irritant-induced laryngospasms. Every parent knows and many have experienced the complications and life-threatening nature of spasmic contraction of the upper airways in a bout of croup. Confounding this speculation is a report of a rail tank car accident in which ammonia was released and a family of four was exposed. Both parents died during the exposure while the two children, although seriously affected, survived [57].

Both acclimation [29,44] and sensitization [75] to ammonia appear to be possible in humans. But, because these two phenomena are only associated with long-term exposure to low levels, they are not likely to have much influence on the probability of acute life-threatening effects of ammonia inhalation.

The various modifying factors discussed above enable broad identification of those within a population who may face greater damage or death from ammonia inhalation (see Table 2-6). Although a somewhat speculative identification, the table does provide guidance on the type of safety factor that will have to be applied, in any risk assessment, to the human LC₅₀ values derived in the next chapter.

TABLE 2-6 Possible Enhanced Risk of Damage or Death from Ammonia Inhalation

<u>Modifying Factor</u>	<u>Sector at Greater Risk</u>
Impaired respiration	* individuals suffering from: - chronic bronchitis - pulmonary edema - cardiovascular disease - chronic allergies - emphysema - respiratory irritation (e.g., common cold)
Sex	* males
Age	* the elderly and young children?

3. DOSE-LETHALITY RELATIONSHIPS

It is not easy to link the results from animal studies and the effects of ammonia exposure on humans. The task requires extensive interpretation and extrapolation. And, as will become increasingly obvious in the discussions that follow, a considerable amount of speculation is involved.

3.1 THE NATURE OF DOSE RESPONSE

The response of a given population (for example, a group of laboratory animals or humans surrounding an ammonia storage facility) to varying doses of a toxicant follows a Gaussian (or normal) distribution. This behaviour is illustrated in Figure 3-1 using mortality due to inhalation of an airborne toxicant as a hypothetical example (dose, in this case, is some function of exposure time and atmospheric concentration of the toxic agent). The dose corresponding to the peak of the distribution is the median lethal dose, a quantity equivalent to the median lethal concentration, LC₅₀, administered for a specified exposure time. The solid line represents behaviour that might be expected from a general population (i.e., a population comprising individuals of all ages and varying health status) -- a distribution which shows a comparatively wide range of response. A sub-group of this population, comprised of young, fit individuals, would exhibit a narrower response range and would have a higher LC₅₀ than the general population (dashed line in Figure 3-1). Integration of the curve produces a cumulative response, (P):

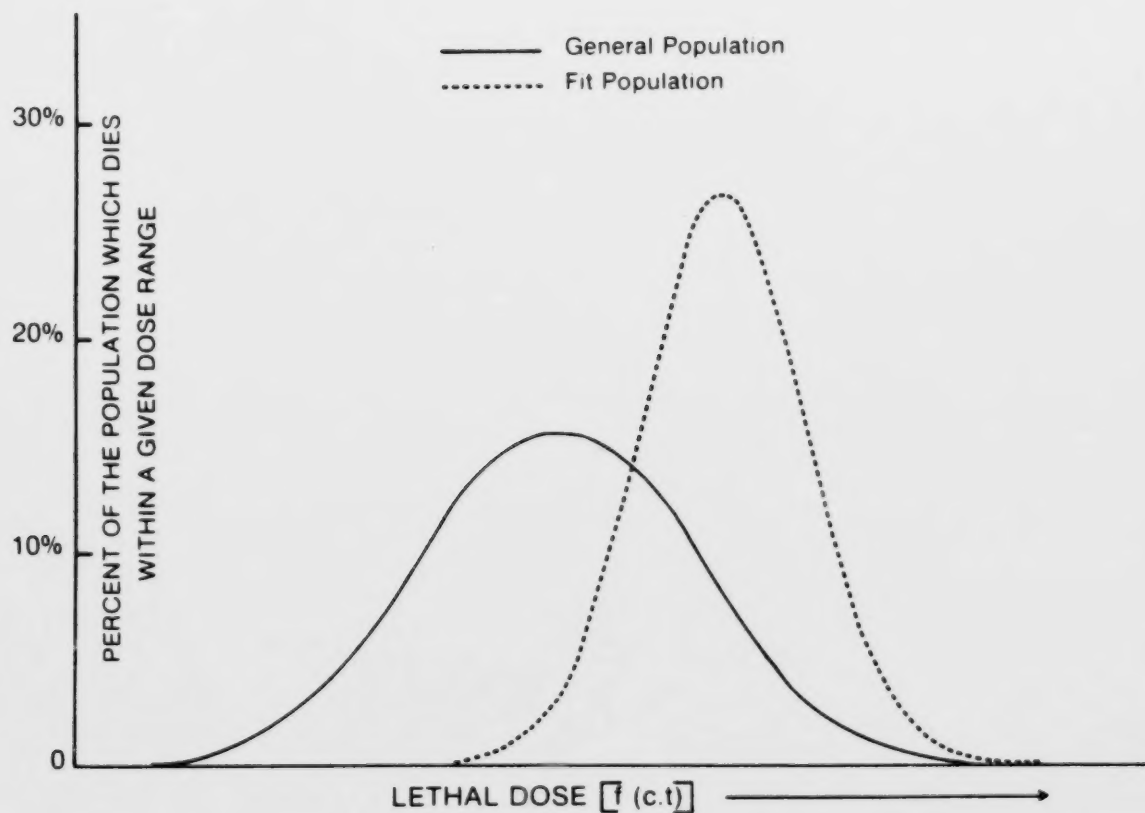
$$(1) \quad P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\gamma-5} \exp^{-1/2 u^2} \cdot du$$

The quantity " γ " is the probability unit or probit and is, itself, a measure of the percentage of an exposed population that exhibits a given response

TABLE 3-1 Relationship Between Cumulative Response and Probit Value*

<u>Cumulative Response</u>	<u>Probit (Y)</u>	<u>Cumulative Response</u>	<u>Probit (Y)</u>
10%	3.72	60%	5.25
20%	4.16	70%	5.52
30%	4.48	80%	5.84
40%	4.75	90%	6.28
50%	5.00	95%	6.64

* Compiled from Table 6-2 of reference [79]

FIGURE 3-1 Examples of Gaussian Distribution in Lethality Response

(e.g., mortality). The probit can be defined as "a Gaussian distributed variable with mean value 5 and variance 1" [reference 79, p. 79]. Its relationship to cumulative response (P) is given by equation (1) and examples of actual values are presented in Table 3-1.

For risk assessment, it is necessary to express toxic response in the form of concentration (c) and time (t) relationships. A number of investigators concerned with risk assessment of ammonia storage have expressed these relationships as probit functions of the following type (see references [77-80] and Section 3.5):

$$(2) \quad \gamma = a \ln (t \cdot c^n) + b,$$

where the coefficients a, b, and n are constants obtained, ideally, from experiment. (The absolute magnitude of LC values predicted by this type of equation is very sensitive to the values of a, b, and n, and these coefficients, in turn, define the width of the response distribution.) However, Griffiths and Megson have cautioned that "the proper application (of probit function relationships) requires the existence of a large body of data on the percentage response to a given exposure" [78]. Because the database from which to derive a human dose-lethality relationship for exposure to ammonia is neither large nor coherent, we have chosen, therefore, to treat the available data using the conceptually simpler relationship:

$$(3) \quad \ln LC_{50} = r \ln t + q;$$

(this relationship is, in fact, a particular solution of equation (2), where $r = 1/n$ and $q = (b-5)/an$). In so doing, it is hoped that the assumptions made in the following sections will be more clearly explicit. However, in the final section of this chapter, a comparison of our dose-lethality relationship

with other investigators' estimates (all of which were expressed as probit functions), a probit function to describe the derived relationship is given.

3.2 DOSE-LETHALITY DATA FROM ANIMAL EXPERIMENTS

There is a comparatively large body of information concerning the effects on animals of short-term high-concentration exposure to ammonia. But in a review of this information (Sections 2.2 and 2.5), only a few studies were found to contain data suitable for the establishment of relationships linking LC_{50} values with exposure time. These data are summarized in Table 3-2 and the lethality/exposure-time relationships suggested by them are presented in Figure 3-2. In choosing these data, preference was of necessity given to those studies which published some detail of the experimental results (instead of citing only an LC_{50} value) and to those which were multi-animal experiments.

As is evident, sufficient information was available only for rats and mice -- less than satisfactory species on which to base predictions of inhalation toxicity in humans. There are no data available for exposure times of less than a few minutes and the response depicted in Figure 3-2 corresponds basically to the Phase II response identified in Section 2.1. The contribution of latent responses (Phases III and IV) to LC_{50} values, from the information presented in Chapter 2, is likely to amount to less than 10 percent and would not be expected to show up in the data plots (in some of the experiments represented in Table 3-2 and Figure 3-2 there was not even an attempt to investigate post-exposure mortality). This small contribution of latent mortality to overall lethality indicates that the excellent fit of Coon and co-workers' long-term data [63] with the results at much shorter exposure times obtained for rats is perhaps more than just fortuitous.

Least-squares linear regression analysis on the data presented in Figure 3-2 yields the following relationships:

for the mouse,

$$(4) \quad \ln LC_{50} \text{ (ppm)} = -0.51 \ln t \text{ (min)} + 10.66$$

with a correlation coefficient of -0.93; and for the rat,

$$(5) \quad \ln LC_{50} \text{ (ppm)} = -0.50 \ln t \text{ (min)} + 11.77$$

with a correlation coefficient of -1.00. The fact that the slopes of the responses are essentially identical for both rodent species and that reasonably good data fits were obtained (despite the variety of experimental methodologies represented) suggests that mammalian response to ammonia exposure is qualitatively similar for different species. The differences between rats and mice (rats appearing to be nearly three times more resistant than mice) probably depend largely on physiological differences (lung size, breathing rate, metabolic rate, and so on), a topic investigated in the following section.

3.3 APPLICABILITY OF ANIMAL MODELS TO THE PREDICTION OF HUMAN RESPONSE

No single species has been identified as a model for the toxicity of inhaled ammonia in humans, and there is no direct analog in laboratory animals to the anatomy and physiology of respiration in humans [7]. But, as argued in the previous section, physiological and related anatomical differences probably play a major part in determining how different species respond. Respiratory differences likely to have some bearing on respiratory toxicity are presented in Table 3-3 and are interpreted below.

On the basis of frequency of respiration (number of breaths per minute at rest), it can be seen that the mouse, the species with the highest respiratory rate, could be most seriously affected by ammonia in the atmosphere because fresh ammonia would be introduced in a greater quantity to

TABLE 3-2 LC₅₀ Data from Animal Experiments*

Reference	Exposure Time (minutes)	LC ₅₀ (ppm)	Sex (# exposed)	Remarks
MOUSE				
[67]	5.0 [†]	24,500 [†]	M (16)	LC ₅₀ values estimated by probit analysis of time-to-death data provided in the paper.
[65]	10	10,170	n.s. (180)	A careful experiment employing a large number of animals; unfortunately the sex breakdown was not given.
[68]	60	4,230	n.s. (36)	A careful experiment employing a large number of animals; unfortunately the sex breakdown was not given.
[8]	120	4,760	n.s. (n.s.)	This LC ₅₀ represents the value chosen by the German National Research Council during their development of occupational-health standards. It represents a composite of a number of research results.
RAT				
[71]	10	40,500	F+M (25+25)	A careful and thorough study to produce estimates of lethality as a function of exposure concentration and time -- specifically designed to be used for risk assessments in the event of rupture of large ammonia storage containers. Probit analysis was used by the paper's authors.
	20	28,700	F+M (25+25)	
	40	20,600	F+M (25+25)	
	60	16,700	F+M (25+25)	
[8]	120	10,930	n.s. (n.s.)	This LC ₅₀ represents the value chosen by the German National Research Council during their development of occupational-health standards. It represents a composite of a number of research results.
[63]	28,800 [†] (20 days)	~750 [†]	F+M (16) [‡]	LC ₅₀ value estimated by probit analysis of the experimental results of long-term (up to 90 d) continuous exposure studies presented in the paper.

* this presentation of lethality data represents neither a complete listing of all such data available nor merely the data most suitable, but rather is a list of the only data found which could be used for the derivation of LC₅₀ vs. time relationships.

n.s. not stated in the paper

[†] these values were obtained by fitting the data presented in the paper to a probit equation of the form:

$$\text{Probit} = a \ln(c^2 \cdot t) + b$$

[‡] the numbers of female and male animals were not specified.

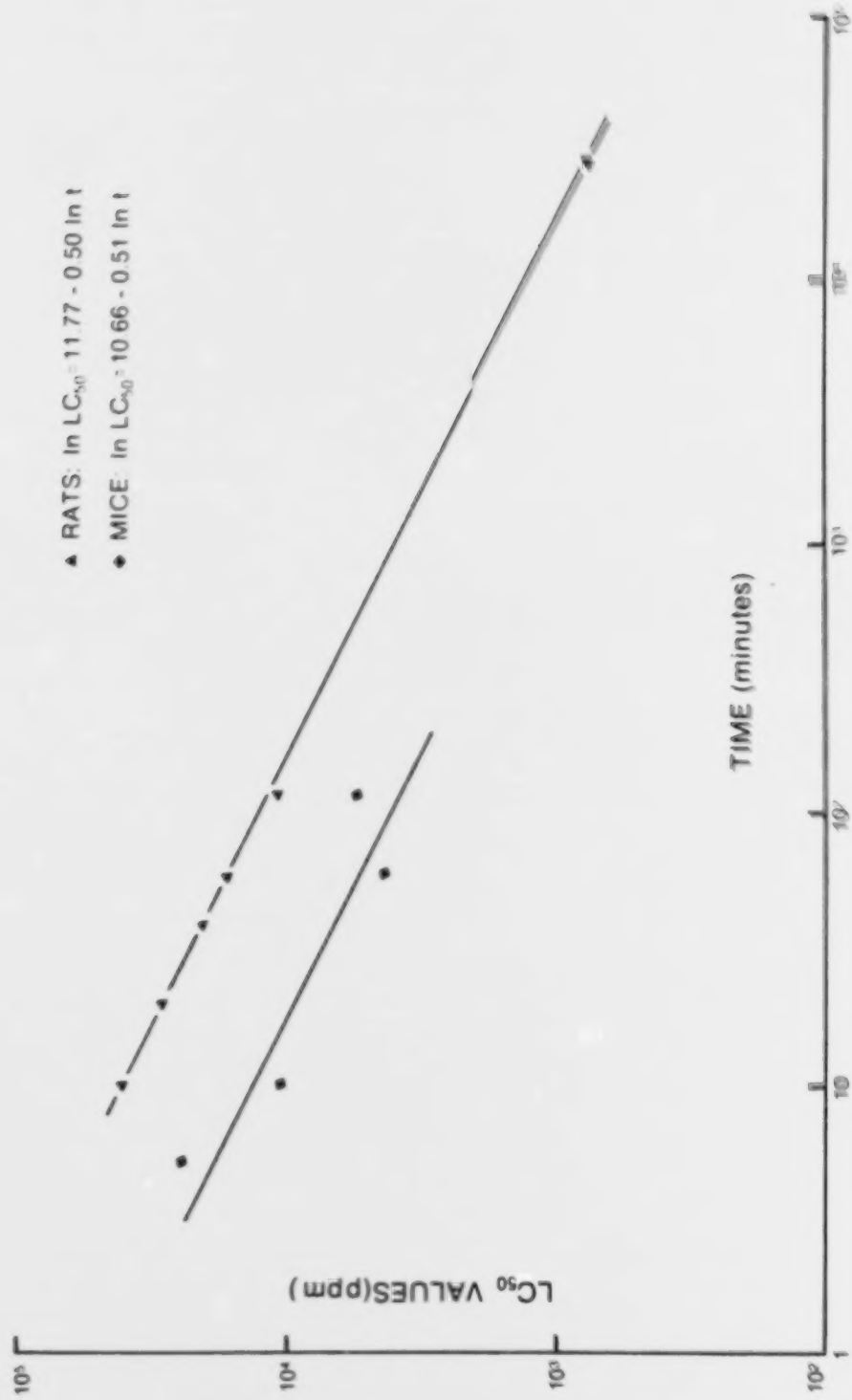


FIGURE 3-2 Median Lethal Concentrations as a Function of Time for the Mouse and Rat

TABLE 3-3 Some Respiratory Indices of Man and Animals*

	<u>Man</u>	<u>Cat</u>	<u>Rabbit</u>	<u>Guinea Pig</u>	<u>Rat</u>	<u>Mouse</u>
Body surface (m^2)	1.8	0.2	0.18	0.040	0.030	0.006
Relation of body surface to body weight (m^2/kg)	0.0257	0.066	0.032	0.12	0.13	0.1
Basal metabolism (k3/kg)	103	==	108	360	613	711
Breaths per min	14-18	20-30	30-100	80-135	110-135	140-210
Size of alveoli (microns)	150	100	==	==	90	30
Surface of lungs (m^2)	50	7.2	5.21	1.47	0.96	0.12
Relation of lung surface to body weight (m^2/kg)	0.7	2.8	2.5	3.2	3.3	5.4
Inhaled air (mL)	616	--	--	1.75	0.865	0.154
Lung ventilation (mL/min)	8732	1000	600	155	73	25
Relation of lung ventilation to body weight (mL/min.g)	0.13	0.30	0.29	0.33	0.05	1.24
Consumption of oxygen (mL/kg.h)	203.1	9420	322.7	2180	2199	3910
Elimination of CO_2 (mL/kg.h)	168.8	--	--	--	2650	4240
Coefficient of respiration	0.82	--	0.83	--	0.82	0.85-1.33
Pulse frequency for 1 min	70-72	120-180	150-180	206-280	300-500	520-780

* From Table 6-2 reference [7]

sensitive lung tissue. And the weight of evidence suggests that mice are more sensitive with respect to lethality than rats and other species (Sections 2.5 and 3.2). It may be more useful still to assess a derived function, for example the factor for lung surface area (m^2) and ventilation (mL/min), which would allow assessment of the rate of irrigation of sensitive lung linings. In this case the order is:

<u>Species</u>	<u>Lung Irrigation Rate (mL/min, m^2)</u>
mouse	208
man	176
cat	138
rat	130
rabbit	115
guinea pig	105

The mouse species thus sees the greatest amount of ammonia impinging a unit area of lung surface, which agrees with its greater sensitivity to ammonia as compared to other species. Man is similar to the mouse with respect to this factor.

The above relationship has relevance only at a plateau phase of exchange, when any difference between inhaled and exhaled ammonia is due only to net absorption into tissues, rather than being the result of dilution of a less contaminated volume of air contained in the lungs. The time required to achieve this saturation is of particular relevance to an interpretation of short-term exposures. In humans, for example, one breath at rest (standard tidal volume, 500 mL) achieves an exchange of only one-seventh of the functional residual volume (3500 mL) in the human lung. Under exercise conditions, this is increased markedly. In either circumstance, however, the initial load of ammonia in lung volumes is always less than the ambient concentration. It would take several minutes of mouth breathing to nearly equal this level in lung air. A valid consideration, however, may be that sudden exposure to a noxious gas such as ammonia may elicit an initial gasp by the victim. This surprise or shock reaction would have the effect of an at least potentially forced inspiration, achieving a more rapid equilibration of lung air with the ambient atmosphere.

None of these relationships between pulmonary variables and ventilation relate to the level of susceptibility for laryngo- or bronchospasm among the species. If an assumption is made that the mucosal receptors of the various test species are similarly responsive to the irritant gas then there may be a direct relationship with the ratio of lung ventilation rate (mL/min) to surface area of the tracheal and bronchial linings. There is not enough information to calculate this, but one can carry out an estimation using the tracheal surface as a target site. First-order approximations for tracheal surface area for human, rabbit, rat, and mouse are 80 cm², 7 cm², 3 cm², and 0.5 cm², respectively. Therefore the sequence for tracheal irrigation of these four species could be:

<u>Species</u>	<u>Tracheal Irrigation Rate (mL/min,cm²)</u>
man	100
rabbit	86
mouse	50
rat	24

On this basis, man appears to be most vulnerable to tracheal effects, and the mouse and rabbit fall in between man and the more resistant rat. Once such tracheal effects occurred, that is tracheospasm, the time to death would be directly dependent on the oxygen requirements of the individual organism. As can be seen from the oxygen consumption data (mL O₂/kg.h) in Table 3-3, this would be the shortest in the cat, suggested to be sensitive to ammonia inhalation, intermediate in the mouse, rat and guinea pig, and longest in rabbit and man. Such an assessment may of course all be spurious if the laryngo-/tracheospasm continues until death. It does mean, however, that the grace period for medical intervention, by way of a tracheostomy, is longest in man. This does correlate with the relative success of this procedure in saving acutely afflicted victims of exposure to ammonia.

It is worth remarking that experimental data presented by Boyd et al. [11] suggest that the one-hour LC₅₀ values for the rabbit and cat are in the region of 5000 ppm, a value close to or slightly lower than that for the mouse. This observation lends a certain credence to the lung irrigation

functions described above, and it can be speculated that mammalian lethality response is some function of the two irrigation rates. To speculate further, however, as to the contribution of each of the two rates to lethality would be a pointless exercise in numerology. Suffice it to say, that the two irrigation functions combined would account, in a general way, for the observed species' differences, and suggest that the human is one of the more sensitive species.

Little can be predicted quantitatively from a consideration of the nasopharyngeal filter. Those species that are nose breathers and have a high surface area in the nasopharyngeal chamber, in proportion to the ventilation rate, would presumably be less critically affected in the early time period of an exposure to ammonia. This includes rats and guinea pigs, which have a proportionately greater nasal surface area as compared to humans and many other species [7].

In summary, it seems that the best model for effects after perhaps several minutes or more of exposure is the mouse, an assumption based both on respiratory parameters and on the fact that a relatively good experimental database exists. The rat is probably less sensitive than man.

It is not really possible to define a similarity between any test species and humans for the earliest phase of exposure, that of nasopharyngeal filtering and irritation. These relationships border on being spurious, and can be used only as a most general guide. What is indicated, however, is that man is probably not uniquely different in any qualitative way from the laboratory animals with regard to the inhalation of ammonia.

3.4 DOSE-LETHALITY RELATIONSHIPS FOR HUMANS

The arguments presented in the previous section suggest that the response of man to ammonia probably lies between that of mice and rats, and may more closely resemble the former than the latter. Mice used in toxicity

studies are commonly healthy young adults (i.e., their response would correspond to the dashed curve in Figure 3-1) and therefore the LC₅₀ values obtained would be greater than those of a "general" mouse population. We propose that the LC₅₀ values obtained from "fit" laboratory mice are also appropriate for the "general" human population -- an admittedly arbitrary assumption.

3.4.1 Exposures Greater Than Five Minutes

Lethality data exist for the mouse only for exposures longer than four or five minutes. Median lethality concentrations (LC₅₀s) corresponding to these exposure times would relate essentially to the Phase II type of response. Although at the higher concentrations corresponding to LC₅₀ values associated with exposure times of less than about 10 minutes, the Phase I laryngospasm response will begin to play a more and more significant role in mortality.

3.4.1.1 LC₅₀ values: Equation (4) of Section 3.2 (the dose-lethality relationship for the mouse) has been chosen to represent the variation with time of LC₅₀ values for humans. This equation implies that for the whole population (males and females combined) the human LC₅₀ varies from about 19,000 ppm at five minutes of exposure to about 3,700 ppm for an exposure of two hours. From the animal experimental data, notably the work of Appelman et al. [71], a significant difference in response between females and males would be expected; with LC₅₀ values for females higher than those suggested by the equation and lower for males. The only estimates of an exposure concentration which resulted in the death of a human (male), 10,000 ppm ammonia for a "few minutes" [51] or 20,000 ppm for half an hour [76], are broadly in accord with these LC₅₀ predictions.

3.4.1.2 LC₁₀ values: In order to estimate LC₁₀ values (the concentration, for a given exposure time, which would result in death of 10 percent of the exposed population) it is necessary, ideally, to have data on the

percentage of deaths occurring in an exposed animal population for a variety of concentrations for a fixed time. (This is particularly important for a toxicant like ammonia, where rapid recovery with few latent systemic effects invariably occurs when the surviving animals are removed from the exposure chamber.) The only experimental data which appear suitable for estimation of LC_{10} values are those of Appelman and co-workers [71]; and these data are for rats rather than mice. These research workers investigated the mortality in groups of animals exposed at five different ammonia concentrations for each of four fixed exposure times (10, 20, 40 and 60 minutes) -- the ammonia concentrations were chosen to bracket the LC_{50} values expected (from preliminary experiments) for each exposure time. Probit analysis was employed to handle the experimental data. Using multiple, linear, weighted regression analysis the authors were able to evaluate the three coefficients in the probit function equation (Equation (2) from Section 3.1) with a considerable degree of confidence. This probit equation gives values for LC_{10} which were 76 percent of the corresponding LC_{50} and demonstrated that the lethality-response distribution is very narrow, at least for the healthy rat.

A broader distribution is to be expected for the general human population. A number of investigators have proposed probit equations for human lethality response to ammonia (see Section 3.5) and these equations variously predict that LC_{10} values are 70 percent (ten Berge and van Heemst [77], Eisenberg et al. [79], COVO2 [81]) or 40 percent (Technica [80]) of the corresponding LC_{50} s. We consider that 70 percent is too high a factor for the "general" population (although it may be appropriate for an adult workforce -- the "fit" population), but that 40 percent is too low (given the narrowness of the distribution experimentally determined by Appelman and co-workers for rats). As an arbitrary compromise, we propose that LC_{10} values for humans be taken as being 50 percent of the corresponding LC_{50} s.

3.4.2 Exposures Less Than Five Minutes

There are no experimental data reported on lethality from exposures to ammonia for periods of less than five minutes, and the question arises as to the validity of Equation (4) at these short exposures. The answer is equivocal.

As was pointed out in Section 3.3, it takes several minutes of mouth breathing for the concentration of ammonia in lung air to approach that of the air being breathed. To highlight, one normal breath of an atmosphere containing 140,000 ppm ammonia (the LC₅₀ predicted by Equation (4) for a 0.1 minute exposure) would result in an initial ammonia concentration in the lung of about 20,000 ppm, a concentration which would be predicted to represent an LC₅₀ for about four minutes of continuous exposure. Clearly, if this were the only factor to be considered, it might be argued that the LC₅₀ curve should be modified as exposure time becomes shorter to produce progressively higher values than those predicted by Equation (4).

On the other hand, because ammonia is so very soluble in water, it may be argued that any ammonia entering the lungs will be immediately absorbed by pulmonary fluids and that, therefore, the body will accumulate ammonia linearly with each breath, at least early in the exposure period before a plateau of uptake is approached. This case may support continued extrapolation of the dose-lethality curve to exposure times as short as six seconds (0.1 minutes).

If these two extreme alternatives were not complication enough, there is the question of laryngo-/tracheospasm. This response occurs demonstrably in humans (and probably in animals too), and is at the same time acutely life threatening (death from asphyxia could occur after about four minutes) and potentially life saving (protection of lung tissues from further damage) if medical aid is immediately available. It is not known what percentage of the population would respond with such spasms at any given concentration, but

is likely that this percentage would rise as exposure concentrations increase. This, coupled with probably increasing damage to epidermal layers, could support the conclusion that the LC_{50} would become progressively lower than that predicted by Equation (4) as exposure times decrease (i.e., a flattening of the dose-lethality curve would be anticipated).

3.4.3 The Human Dose-Lethality Curve

We have hypothesized that human lethality response to ammonia inhalation is, of the test species for which there are credible experimental data, mimicked most closely by the mouse. Accordingly, for exposures from two hours to five minutes, LC_{50} values would be calculated using Equation (4). Taking into account the arguments presented in the previous section, we feel that on balance there is likely to be a flattening of the human dose-lethality curve at exposures shorter than five minutes. The extent of this flattening is uncertain. The effect of the non-equivalence of ammonia concentrations in lung and ambient air has to be offset by the probable increase in tendency for laryngospasm and the cumulative dose effect due to ammonia solubility. A risk assessment model, however, requires definite numbers: we therefore speculate that from five minutes to two minutes these factors approximately cancel out and that, for modelling purposes, the curve be linearly extrapolated. For exposures less than two minutes, we propose that the LC_{50} value be considered constant. Following the arguments presented in Section 3.4.1.2, we propose, in addition, that LC_{10} values be considered to be 50 percent of the equivalent LC_{50} values. Examples of the predicted LC_{50} and LC_{10} values are presented in Table 3-4. It cannot be emphasized enough, however, that these dose-lethality curves for humans are based on few data and considerable speculation -- they should be used predictively only with appropriate circumspection.

The risk assessment model used by the Atomic Energy Control Board [5] requires that the variation of LC_{50} values with exposure time be expressed as a step function. There is really very little quantitative evidence (except,

TABLE 3-4 Predicted LC₅₀ and LC₁₀ Values for Humans

Time	LC ₅₀ [*] (ppm)	LC ₁₀ [†] (ppm)
2 hrs	3,700	1,900
1 hr	5,300	2,600
30 min	7,500	3,800
10 min	13,000	6,600
5 min	19,000	9,400
2 min	30,000	15,000
<2 min [‡]	30,000	15,000

* predicted by Equation (4)

† assumed to be 50% of the LC₅₀ (see Section 3.4.2)‡ at exposures from 6 to 120 seconds, LC₅₀ is assumed to be constant

Note: Because of the paucity of data upon which all these values are based, the lethal concentrations are only expressed to two significant figures.

TABLE 3-5 Step-Function for Human LC₅₀ Values

Exposure Time (min)	LC ₅₀ (ppm)
0.1 to 2.0	30,000
2.0 to 4.3	25,000
4.3 to 9.3	17,000
9.3 to 20	11,000
20 to 43	7,600
43 to 93	5,100
93 to 200	3,500

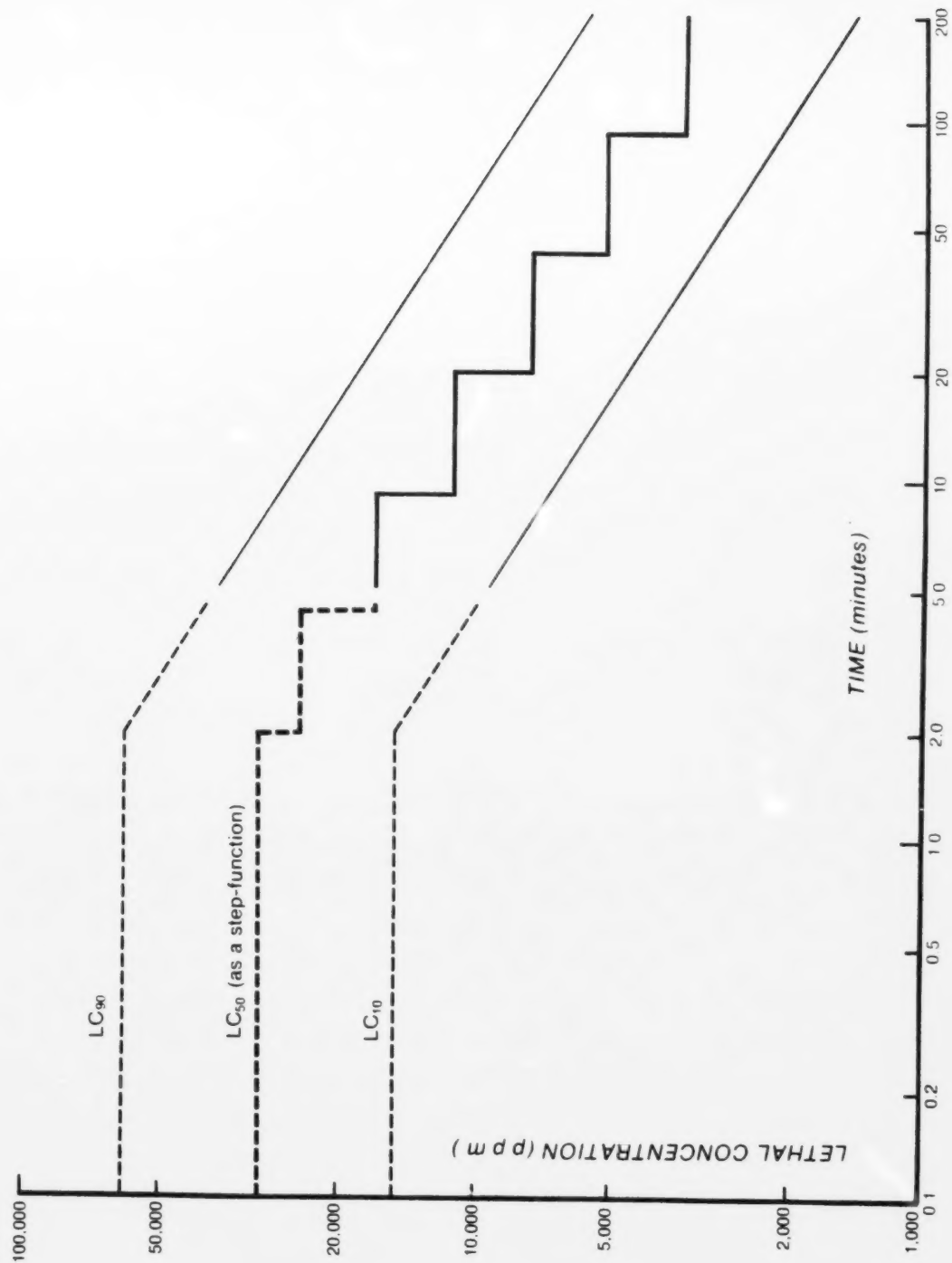


FIGURE 3-3 Human LC_{50} as a Step-Function with Respect to Time

perhaps, at the shortest exposures) that a multi-phase response exists for mortality due to acute ammonia inhalation, certainly not for exposures up to two hours. The assumption of a four-phase longer-term response cannot be quantified for the purposes of risk assessment in this exercise. Accordingly we suggest that the dose response curve be "stepped" at no less than three increments per decade in time. The result of applying three equal increments per decade on LC_{50} values is shown in Table 3-5 and illustrated in Figure 3-3. Also shown in Figure 3-3 are LC_{10} and LC_{90} values (one half and twice the LC_{50} values, respectively) to give an idea of the breadth of the lethal response distribution that is being proposed.

3.5 COMPARISON OF DOSE-LETHALITY RELATIONSHIPS PREDICTED FOR HUMAN EXPOSURE TO AMMONIA

In recent years, a number of attempts have been made to estimate dose-lethality relationships for human exposure to ammonia. These are summarized in Table 3-6 and illustrated in Figure 3-4. Perhaps the most remarkable feature is that the various estimates all agree within a factor of about three or four. In particular, our estimates and those of Technica [80] and COVO2 [81] can be considered to be essentially the same, even though the routes by which they were derived differed. Technica based their estimate on two published LC_{50} values for the mouse (those of Silver and McGrath [65] and Kapeghian et al. [68]) and one, unfortunately misinterpreted, value for the rat (that provided by the data of Carpenter et al. [70]). The COVO2 derivation [81] equated the mortality response in humans "to that of the average experimental animal" but did not identify the animal data from which the results were obtained. The estimate of ten Berge and van Heemst [77] was based on the excellent experimental data that Appelman and co-workers [71] obtained for the rat. The coefficients of the probit equation were "decreased more or less arbitrarily" by ten Berge and van Heemst to yield an equation more appropriate to the general human population than that of the "fit" experimental animal. The estimate that is furthest from the others depicted in Figure 3-4 is that of Eisenberg et al.

[79]. As far as we know, theirs was the first attempt to use probit analysis in the prediction of human lethality responses to ammonia. The concentration coefficient ("n" in Equation (2)) was assumed by Eisenberg and colleagues to be the same as that observed for chlorine exposure, namely 2.75 instead of the 2 used by later investigators. The probit function was based on scant data obtained from reference textbooks on hazardous compounds, rather than on published experimental work.

Our own dose-lethality relationship, when treated as a special case of the probit function (i.e., as in Equation (3) in Section 3.1), has a concentration dependence of 1.96 (1/0.51), essentially the same as the factor of 2 used by ten Berge and van Heemst [77], Technica [80] and COVO2 [81] and experimentally determined in rats by Appelman *et al.* [71]. There seems little doubt, therefore, that 2 is the appropriate concentration coefficient to use for mammalian exposure to ammonia.

The data we predicted for LC₅₀ and LC₁₀ values for humans (shown in Table 3-4) can be generated, with very close agreement, by the following probit function:

$$(6) \quad \gamma = 0.94 \ln (t \cdot c^2) - 15$$

where t = time in minutes and c = ammonia concentration in ppm. The caveats mentioned in Section 3.4.3 apply to its predictive use.

TABLE 3-6 A Comparison of Predicted LC₅₀ Values for Human Exposure to Ammonia*

Exposure Duration (min)	ten Berge and van Heemst (1983) [77]	COV02 (1982) [81]	This Report	Technica (1984) [80]	Eisenberg et al. (1975) [79]
5	29,000	20,000	19,000	15,000	6,300
10	20,000	14,000	13,000	11,000	4,900
30	12,000	8,100	7,500	6,200	3,300
60	8,300	5,800	5,300	4,400	2,600
120	5,900	4,100	3,700	3,100	2,000

* All values quoted have been rounded to two significant figures.

Lethality relationships from which the above values were calculated:

ten Berge and van Heemst [77]: Probit = $1.9 \ln (t \cdot c^2) - 37.1$

COV02 [81]: Probit = $1.91 \ln (t \cdot c^2) - 35.9$

This report: $\ln LC_{50} = 10.66 - 0.51 \ln t$

Technica [80]: Probit = $0.71 \ln (t \cdot c^2) - 9.82$

Eisenberg et al. [79]: Probit = $1.385 \ln (t \cdot c^{2.75}) - 30.57$

where t = time in minutes and c (or LC₅₀) = ammonia concentration in ppm.

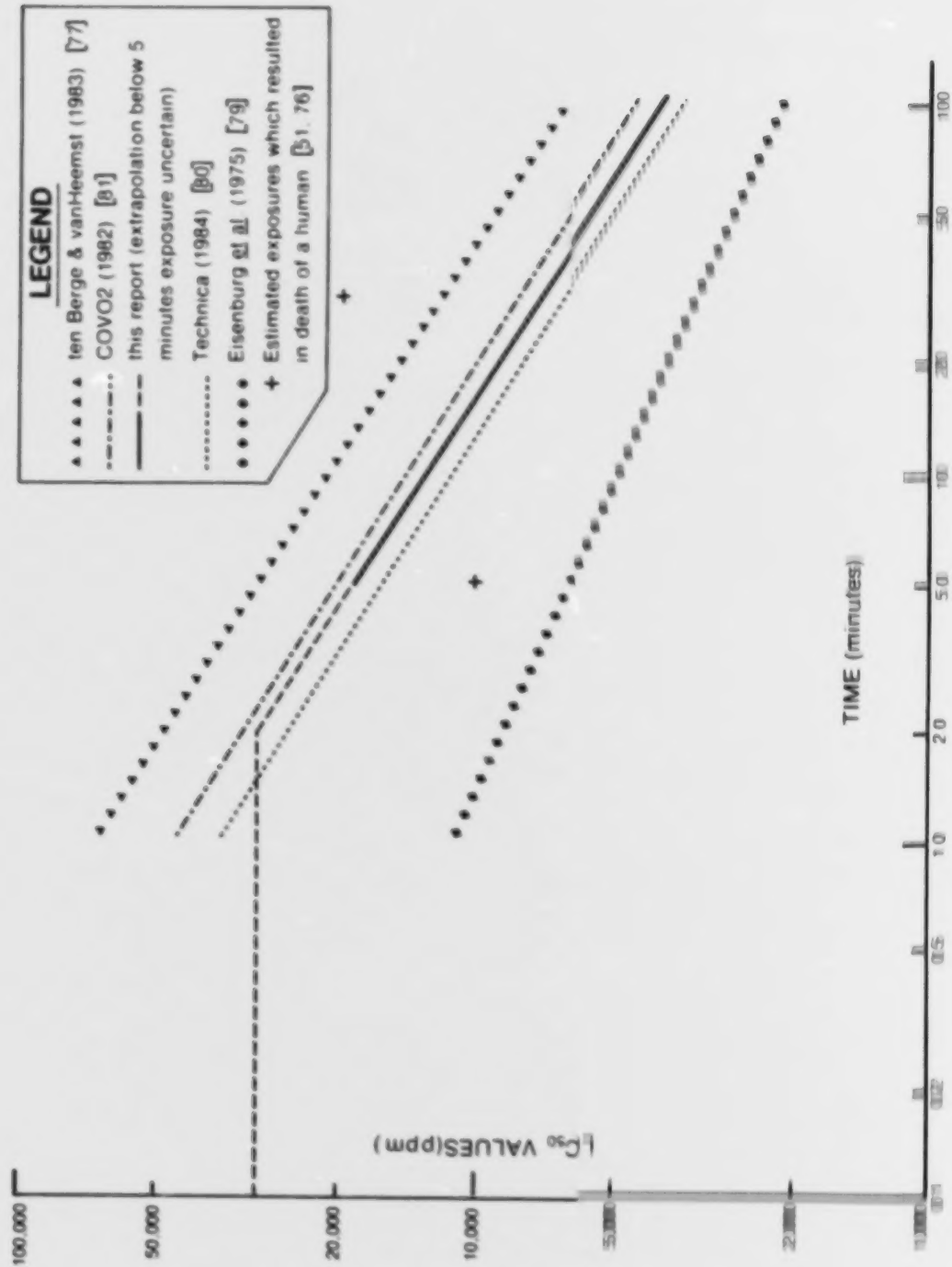


FIGURE 3-4 Various Dose-Lethality Relationships Proposed for Human Exposure to Ammonia

4. SUMMARY

This report has focussed on the derivation of dose-response relationships in humans at exposures from six seconds to two hours. Ammonia, a highly alkaline and highly irritating gas, exerts its noxious effects primarily on the respiratory system, although skin, eyes, and the nervous system may also be affected by exposure to high atmospheric concentrations. The poisonous nature of the gas has been long recognized and was reported two and a half centuries ago, and has been identified as the cause of accidental death and injury many times since then. While the mode of action of ammonia has been well described in the case histories, there are no data for concentrations and even durations of the short exposures which were found to be harmful.

The development of a lethality index for humans has of necessity been derived from knowledge of the physical and chemical properties of the gas and from interpretation of its dose-response relationships in animal studies. It is fortunate in this respect that the case histories for accidental exposures demonstrate a toxicity in humans which is qualitatively similar to that observed in experimental studies using laboratory animals.

With respect to physio-chemical properties, ammonia is a gas at room temperature but will become a liquid when under pressure and/or when cooled, as for instance in refrigeration systems or in storage and transport facilities. Most of the accidents have been the result of a sudden rupture of pressure vessels. Ammonia is also extremely soluble in water, forming ammonium hydroxide, and this results in a very fast absorption into the body from the air through the nasopharyngeal filter and pulmonary membranes; the hydroxylated form is readily excreted.

Expression of the toxicity of ammonia depends on concentration and duration of exposure. An odour threshold occurs at 1 to 50 ppm, well below concentrations that produce observable toxicity. A threshold of unbearable

discomfort is reached, in most people, at approximately 500 ppm, and is marked by strong irritation of the eyes and of the upper respiratory tract. Acute constriction of upper respiratory passages (laryngo- and bronchospasms) may occur in some individuals at 2000 ppm. Higher levels at ever decreasing durations of exposure result in pulmonary edema, decreased respiratory capacity, and increased risk of death. At such high concentrations of ammonia, a four-phase lethality response may be identified:

- 1 Phase I = acute spasmodic obstruction of upper airways
= occurs within seconds to minutes during exposure
- 1 Phase II = massive pulmonary edema and extravasation,
sloughing of epidermal layers
= occurs in the minutes during exposure and for several
hours post-exposure
- 1 Phase III = bacterial pneumonia
= may occur within a week to two months post-exposure
- 1 Phase IV = progressively obstructive lung disease
= may occur months to years after exposure

The probability of death associated with each of the first three phases can be decreased by effective medical treatment (e.g., tracheostomy in phase I). Phase IV fatality appears to be a rare phenomenon in ammonia poisoning.

The circumstances of accidental exposure to ammonia are varied, but usually involve the rupture of a pressurized system, exposing individuals in the immediate vicinity to high atmospheric concentrations. The majority of fatalities occur under such circumstances, although the actual doses are not quantifiable. For a so-called "high" and fatal (Phase II) exposure, levels have been variously estimated at 10,000 or 20,000 ppm. The overall historical fatality index, drawing on all available reported accidental exposures, has been calculated by us to be 18 percent: 44 deaths among 251 exposed individuals. This, however, does not account for differences in exposure levels and duration, nor for the fact that there were many reports of tracheostomies carried out to relieve respiratory distress due to Phase I response.

Certain sectors of a randomly exposed population may be at greater risk than others. Presumably people in poor respiratory health, suffering perhaps from edema or emphysema, would be more severely affected by acute exposure to ammonia. Experimental data showed female rats and mice to be more resistant to the lethal effects of ammonia than their male counterparts in the test populations. This relationship could not be verified from the human data. Although one may assume age to be an influential variable, no conclusion can be drawn about the direction of the effect on expression of ammonia toxicity.

Ammonia studies have generally verified the target sites of ammonia poisoning. A progression of levels of tissue involvement is indicated, from the initial irritation of skin, eyes, nose, mouth, and throat, to irritation and burning of larynx, trachea, and bronchi, and then to the lower respiratory tract. Among the six species for which there are data on acute toxicity (mouse, rat, guinea pig, rabbit, cat, and bat) only a few mouse and rat experiments were found to be of a design and validity to allow construction of LC_{50} versus exposure time relationships. Least-squares linear regression analysis of the available data yielded the following relationships: for the mouse,

$$(4) \quad \ln LC_{50} \text{ (ppm)} = -0.51 \ln t \text{ (min)} + 10.66$$

and for the rat,

$$(5) \quad \ln LC_{50} \text{ (ppm)} = -0.50 \ln t \text{ (min)} + 11.77$$

Good data fits were obtained and the slopes for both species are essentially identical. Rats appear to be nearly three times more resistant than mice.

Considering respiratory anatomy and physiology, no direct counterpart for humans exists among laboratory animals. An exercise was carried out which tried to identify animal models for the human condition with respect to

respiratory functions critical to the expression of acute ammonia toxicity. Based on a derived function -- lung irrigation rate (mL air/min.m^2) -- the mouse data were considered to be more representative of pulmonary effects in humans than those of the rat.

The LC_{50} relationship for the mouse (Equation (4)) was chosen to model the human mortality response at exposure times between two minutes and two hours. This would predict for the human population that LC_{50} values vary, for example, from about 19,000 ppm at five minutes exposure to about 3,700 ppm for an exposure of two hours. (Males may be more sensitive than females to the lethal effects of exposure to ammonia.) There is increasing uncertainty about the slope of the dose-lethality curve (plotted as the logarithm of LC_{50} versus time) for exposure durations less than about five minutes. It was rationalized that the slope of the curve should progressively flatten at shorter exposures and even become zero at very short exposure times. There is no way of quantifying this decrease in slope, so it was assumed that, for risk assessment purposes, LC_{50} values for exposures from five minutes to two minutes are those given in Equation (4), but that at shorter exposure times the LC_{50} should be considered constant (at 30,000 ppm). The LC_{10} values for humans were suggested, more or less arbitrarily, to be half that of the corresponding LC_{50} s. The LC_{50} values predicted by Equation (4) are in good agreement with those recently derived for human exposure to ammonia by other investigators. These other workers expressed the variation of lethal concentration with time in the form of a probit function: an approach that is gaining increasing acceptance in risk assessment. (The advantage of a probit equation is that, theoretically, lethal concentrations for other than 50-percent mortality can be obtained.) The LC_{50} and LC_{10} values predicted by our human dose-response model (for exposures greater than two minutes) can be closely approximated by the probit function:

$$(6) \quad \gamma = 0.94 \ln (t \cdot c^2) - 15$$

where t = time in minutes and c = atmospheric concentration in ppm.

A final note to this exercise in extrapolation and conjecture is that the dose-lethality relationships for humans are based on few data and considerable, although reasoned, speculation. The product should be used predictively only with appropriate circumspection.

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